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HOG'S GASTRIC MUCIN AS AN AID IN THE ISOLATION OF *N. GONORRHOEAE* *†

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A diagnosis of gonorrhoea can only be made with certainty by the isolation of *N. gonorrhoeae*. The reliance for diagnosis on stained films alone has been shown by De Bord (1939), Ewing (1949), Schaub and Hauber (1948), and others to be too uncertain to be accepted as proof of infection. Moreover, cultural methods yield a higher proportion of positive results, especially in chronic cases (McLeod, Coates, Happold, Priestley, and Wheatley, 1934; Eldering and Palser, 1946).

Unfortunately, the gonococcus is often very difficult to cultivate from pathological material, and it is certainly the most difficult member of the Neisseria group to isolate. Many factors have to be carefully considered, *e.g.*, *p*H and consistency of medium, temperature, carbon dioxide content of environment, and so on. While these conditions can be fulfilled in large central bacteriological departments, they may place a heavy burden upon the personnel of smaller subsidiary laboratories. Again, clinics may be sited some distance from the parent laboratory, and the bacteria may not survive transportation.

It would, therefore, be of advantage to have a medium which could maintain the organism in transport over long distances with fluctuations in temperature *en route*. The essence of such a medium should be that it is simple to prepare and easy to handle. Many substances have been advocated for their growth-promoting action towards *N. gonorrhoeae*. Blood, serum, and ascitic fluid have long been regarded as essential ingredients of media, and an atmosphere of carbon dioxide is usually regarded as necessary for satisfactory cultivation. It was reported by Glass and Kennet (1939) that charcoal (heated to 1,000 C. to destroy organic matter) could replace blood. Starch has been used by different workers (Gould, Kane, and Mueller, 1944; Peizer, Steffen, and Klein, 1949). Media have been suggested for maintaining the gonococcus in transport. Cooper, Mayr-Harting, and McLachlan (1950), used a crystal violet blood mixture and later thallium acetate blood mixtures; Moffett, Young, and Stuart (1948), used thioglycollic acid, sodium glycerophosphate, calcium chloride, and methylene blue; Stuart (1946) used a non-nutrient medium with sodium thioglycollate; and Eldering and Palser (1946) used proteose No. 3 agar and haemoglobin medium (Difco).

Most of the media suggested are rather complex, and therefore a simpler medium with less rigorous requirements of cultivation would be an advantage. While carrying out studies on the growth-promoting action of hog's gastric mucin towards the meningococcus (Maccabe and King, 1951) one wondered what effect mucin might have on the growth of another member of the Neisseria group—*N. gonorrhoeae*.

This paper illustrates the effect of mucin on the growth of the gonococcus, using, in the first instance, pure cultures of the organism and, secondly, pathological material from cases.

Media

Mucin.—The mucin used was granular mucin Type 1701–W (Wilson and Company, Chicago). A 3 per cent. suspension in sterile distilled water was shaken by hand for 20 min. to suspend particles, and sterilized at 100°C. for 20 min. It was then standardized to *p*H 7·4 by addition of sterile phosphate buffer M/5 Na_2HPO_4.

Medium for Growth

Agar	1·4 per cent.
Peptone	0·7 per cent.
Laboratory Lemco	0·7 per cent.
Sodium chloride	0·35 per cent.
Mucin	0·64 per cent.
Defibrinated horse blood	9 per cent.

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The medium was inoculated cold with pure culture or exudate and incubated aerobically at 37°C. with and without carbon dioxide. This medium is despatched on petri plates.

Medium for Preservation in Transport.—This medium is despatched in small screw-capped bottles containing:

Crude mucin 3 per cent. . .	. . 1·5 ml.
Defibrinated horse blood	. . 1·5 ml.
Crystal violet concentration	. . 1:1,000,000

Specimens were taken on sterile swabs. The swab was immersed in the medium, the swab stick cut off at the level of the neck of the bottle, and the screw cap applied. The bottles were left for 24, 48, and 72 hrs at temperatures of 60–65°F. and 45–50°F. before subculture.

Results

Table I summarizes the results of several experiments using varying dilutions of pure cultures as the inoculum.

The growth was judged by the number and size of the colonies, and this was confirmed by harvesting the whole growth from each place and estimating turbidity.

TABLE I

GROWTHS OF *N. GONORRHOEAE* ON ORDINARY BLOOD AGAR AND BLOOD AGAR WITH MUCIN, AFTER 24 HOURS AT 37°C.

Dilution of Culture	Blood Agar		Blood Agar + Buffer		Blood Agar + Mucin	
	CO_2	No CO_2	CO_2	No CO_2	CO_2	No CO_2
1 in 10	++	±	++	±	++++	+++
1 in 100	+	−	+	−	+++	++

− = no growth. + = slight. +++ = profuse.
± = very slight. ++ = moderate. ++++ = very profuse.

Table I illustrates that:

(1) Growth is more profuse on mucin medium;
(2) In some cases CO_2 was not required when using the mucin medium.

It was also observed that:

(1) Growth appeared sooner on mucin media in all cases;
(2) The colonies on the mucin medium were much larger than on ordinary routine media;
(3) In some cases an old culture which failed to grow on blood agar and CO_2 was made to survive after incubation on mucin medium.

A refined fraction of mucin was used in the above experiments and at a concentration of 0·03 per cent. gave results similar to the crude product.

Table II shows that a mixture of mucin and blood can maintain the gonococcus for 24 hrs at 45–50°F. in all cases, and for 48 hrs in 82 per cent. of cases, whereas neither mucin nor blood was sufficient alone. Mucin and blood never failed to maintain growth from all cases with positive films.

TABLE II

PERCENTAGE OF SPECIMENS FROM 25 PATIENTS WITH GONORRHOEA WHICH GAVE POSITIVE CULTURES

Hours	Temperature (°F.)	Transporting Medium		
		Mucin (per cent.)	Blood (per cent.)	Mucin and Blood (per cent.)
24	60–65	0	27	100
	45–50	0	0	100
48	60–65	0	0	82
	45–50	0	0	82
72	60–65	0	0	0
	45–50	0	0	0

Table III illustrates the growth of the gonococcus from pathological material on a blood agar medium with mucin added, without addition of carbon dioxide.

TABLE III

PERCENTAGE OF SPECIMENS FROM 25 PATIENTS WITH GONORRHOEA WHICH GAVE POSITIVE CULTURES, WITHOUT CO_2

Blood Agar (per cent.)	Mucin Blood Agar (per cent.)
0	76

Summary and Conclusions

(1) This work has shown that hog's gastric mucin enhances the growth of *N. gonorrhoeae*. In most cases carbon dioxide is not necessary when employing a medium containing mucin. The stimulating effect of carbon dioxide on certain bacteria is not fully understood. Recent work has suggested that it is essential for the synthesis of certain compounds, *e.g.*, the C_4 dicarboxylic acids essential for growth of the organism. It may contribute to the synthesis of an accessory growth factor, provide an additional source of carbon, or neutralize toxic products in the medium (Glass and Kennet, 1939; Bigger and Nelson, 1943; Wherry and Ervin, 1918; Rockwell and McKhann, 1921; Hes, 1938; Gladstone, Fildes, and Richardson, 1935; Wood, Werkman, Hemingway, and Nier, 1940; Pappenheimer and Hottle, 1940; Krebs and Eggleston, 1941). Whether mucin acts in this manner is not known.

(2) The gonococcus can be maintained for at least 48 hrs in a mixture of blood and mucin, which facilitates its isolation from pathological material, when laboratories are some distance from the case.

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